

Biofilm production and enzyme activity of *Candida* strains isolated from patients with dental braces

Paweł Krzyściak¹, Kinga Grzegocka², Patrycja Biedrzyńska³, Anna Lipiarz³, Katarzyna Talaga¹, Bartłomiej Loster²

¹Chair of Microbiology, Department of Mycology, Jagiellonian University Medical College, Czysła 18, 31-121

Kraków; ²Chair and Department of Orthodontics, Dental Institute, Jagiellonian University Medical College,

Montelupich 4, 31-155 Kraków; ³Students of Biotechnology, Faculty of Biotechnology and Horticulture, University of Agriculture, Mickiewicza Av. 21, 31-120 Kraków, Poland

Corresponding Author: Paweł Krzyściak; e-mail: pawel.krzyściak@uj.edu.pl

Orthodontic treatment can significantly affect the population of *Candida* in the oral cavity. Complicated construction of permanent hearing make generally hard the oral hygiene. Few studies, focusing on the presence and role of the bacteria on the condition of the enamel, indicate the role of elastic ligatures in increasing the number of microorganisms in the oral cavity.

The aim of this study was to determine characteristics of fungi isolated from patients using orthodontic braces. One hundred fourteen materials (oral washings and dental ligatures) collected from 17 patients were tested. The washings and ligatures were plated on Sabouraud agar and incubated for 72 h at 37°C. Yeast was identified based on its ability to produce chlamydospores and by API 20C AUX test.

The biofilm formation assay was performed on sterile 96-well flat-bottom polystyrene plates with Yeast Nitrogen Broth supplemented with 0.2% glucose. After 72 h of incubation formed biofilm was staining with crystal violet and after decolorization absorbance were measured. Each strain was analyzed in 9 replicates. Phospholypolytic and proteolytic activity was detected on Egg Yolk and Bovine Serum Albumin agar respectively by measuring the ratio of the diameter of colony and precipitation or translucent halo zone (Pz). Analyzes were performed in duplicate. The study included 28 isolates and reference strain of *C. albicans* (ATCC 90028). A positive culture was obtained from 50 materials of 12 patients: 18 elastic ligatures and 32 oral washings. The dominant species was *C. albicans*, furthermore single strains of *C. tropicalis* and *C. guilliermondii* was isolated. Biofilm formed 89.3% investigated strains. 18 strains formed biofilm greater or comparable to that biofilm formed by the reference strain; 7 strains produced lower biofilm and 3 didn't produce any biofilm.

The average phospholypolytic activity (expressed as Pz) was equal to 0.71 ± 0.18 , range 0.32–1.00, however 5 strains didn't grow on used medium. Proteolytic activity was comparable for all tested strains. The average Pz value was 0.61 ± 0.08 with range 0.48–0.79 (one strain didn't hydrolyze albumin). Strains isolated from patients using orthodontic braces have a greater ability to form biofilm than the reference strain. There is no relationship between the proteolytic and phospholypolytic activity and the ability of biofilm formation by strains isolated from patients using braces.